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Morphological and Genetic Characteristics of *Colletotrichum gloeosporioides* Isolated from Newly Emerging Static-Symptom Anthracnose in Apple

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Filamentous fungi of the genus *Colletotrichum* (teleomorph, *Glomerella*) are considered major plant pathogens worldwide. Cereals, legumes, vegetables, and fruit trees may be seriously affected by this pathogen (1). *Colletotrichum* species cause typical disease symptoms known as anthracnoses, characterized by sunken necrotic tissue, where orange conidial masses are produced. Anthracnose appears in both developing and mature plant tissues (2). We investigated disease occurrence in apple orchards from 2013 to 2014 in northern Gyeongbuk province, Korea. Typical anthracnose with advanced symptoms was observed in all apple orchards studied. Of late, static fruit spot symptoms are being observed in apple orchards. A small lesion, which does not expand further and remains static until the harvesting season, is observed at the beginning of fruit growth period. In our study, static symptoms, together with the typical symptoms, were observed on apples. The isolated fungus was tested for pathogenicity on cv. 'Fuji apple' (fully ripe fruits, unripe fruits, and cross-section of fruits) by inoculating the fruits with a conidial suspension (10^5 conidia/ml). In apple inoculated with typical anthracnose fungus, the anthracnose symptoms progressed, and dark lesions with salmon-colored masses of conidia were observed on fruit, which were also soft and sunken. However, in apple inoculated with fungi causing static symptoms, the size of the spots did not increase. Interestingly, the shape and size of the conidia and the shape of the appressoria of both types of fungi were found to be similar. The conidia of the two types of fungi were straight and cylindrical, with an obtuse apex. The culture and morphological characteristics of the conidia were similar to those of *C. gloeosporioides* (5). The conidia of *C. gloeosporioides* germinate and form appressoria in response to chemical signals such as host surface wax and the fruit-ripening hormone ethylene (3). In this study, the spores started to germinate 4 h after incubation with an ethephon suspension. Then, the germ tubes began to swell, and subsequently, differentiation into appressoria with dark thick walls was completed by 8 h. In advanced symptoms, fungal spores of virtually all the appressoria formed primary hyphae within 16 h. However, in the static-symptom fungus spores, no primary hyphae formed by 16 h. The two types of isolates exhibited different growth rates on medium containing apple pectin, Na polypectate, or glucose as the sole carbon. Static-symptom fungi had a >10% reduction in growth (apple pectin, 14.9%; Na polypectate, 27.7%; glucose, 10.4%). The fungal isolates were also genetically characterized by sequencing. ITS regions of rDNA, chitin synthase 1 (CHS1), actin (ACT), and β-tubulin (βt) were amplified from isolates using primer pairs ITS 1 and ITS 4 (4), CHS-79F and CHS-354R, ACT-512F and ACT-783R, and T1 and βt2 (5), respectively. The resulting sequences showed 100% identity with sequences of *C. gloeosporioides* at KC493156, and the sequence of the βt gene showed 100% identity with *C. gloeosporioides* at JX009557.1. Therefore, sequence data from the four loci studied proves that the isolated pathogen is *C. gloeosporioides*. We also performed random amplified polymorphic DNA-PCR, which showed clearly differentiated subgroups of *C. gloeosporioides* genotypes. The clustering of these groups was highly related to the symptom types of the individual strains.

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